Rapid Profiling of Saccharides Using HPLC with ELSD for Improved Precision

Application Note

Food

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Introduction

Saccharides are of great importance in nature, and chemists and biochemists require sensitive and robust analytical methods for their identification and quantification.

These compounds do not possess a UV chromophore and are therefore not suited to UV detection. Normally, the nonchromophoric sugar separations would be performed using a refractive index (RI) detector. However, RI commonly suffers from baseline instability and poor sensitivity. Due to the nonvolatile nature of saccharides, evaporative light scattering detection (ELSD) using the Agilent ELSD, is better for this type of analysis, offering excellent baseline stability. There are a number of HPLC methods to quantify saccharides, with one of the most simple being the use of a calcium ligand-exchange column, Agilent Hi-Plex Ca, with water as the eluent.

The Hi-Plex Ca column contains a monodisperse sulphonated polystyrene incorporating 8% divinylbenzene with a calcium counter ion and provides a separation based on a combination of both size exclusion and ligand-exchange chromatography. These soft gel columns are operated at elevated temperature in order to reduce operating pressure and permit regular flow rates to be employed. Sensitivity in saccharide detection is achieved by using the Agilent ELSD. In addition to an increase in sensitivity, this detector also gives a more stable, drift-free baseline, improving the precision of the quantitation.

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Experimental

Instrumentation

Column	Agilent Hi-Plex Ca, 7.7 × 300 mm, 8 µm (p/n PL1170-6810)
Detector	Agilent ELSD

Materials and Reagents

Mobile phase 100% DI H_2O

Sample Preparation

Saccharides were dissolved in water at 1.0 mg/mL.

Results and Discussion

Figure 1 shows that the five saccharide standards are well-resolved and the baseline is extremely stable.

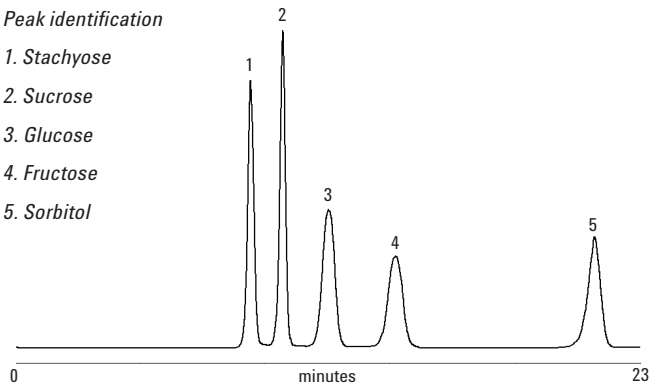

Figure 1. Excellent separation to baseline of five saccharides by Agilent Hi-Plex Ca columns with the Agilent ELSD.

Conclusion

Combining the Agilent Hi-Plex Ca column with the Agilent ELSD provides an excellent solution for resolving saccharides. The sulfonated resin in Hi-Plex Ca offers a fundamental improvement in performance. Its monodisperse sulfonated packing overcomes problems of low efficiencies and high backpressures encountered with soft gels. The Agilent ELSD surpasses other ELSDs for low-temperature HPLC applications with semivolatile compounds. The Agilent ELSD's unique gas control permits evaporation of high boiling solvents at very low temperatures. For example, 100% water at a flow rate of 5 mL/min can be removed at 30 °C.

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